

Microbial cycling of methanethiol

Hendrik Schäfer¹ and Özge Eyice²

¹ School of Life Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom

² School of Biological and Chemical Sciences, Queen Mary University of London, E1 4NS, London, United Kingdom.

Running title: Microbial cycling of methanethiol

For correspondence:

Email: H.Schaefer@warwick.ac.uk

Abstract

Methanethiol (MT) is an organic sulfur compound with a strong and disagreeable odor. It has biogeochemical relevance as an important compound in the global sulfur cycle, where it is produced as a reactive intermediate in a number of different pathways of production and degradation of other globally significant sulfur compounds such as dimethylsulfoniopropionate, dimethylsulfide and methionine. With its low odor threshold and unpleasant smell, methanethiol can be a significant cause of malodor that from animal husbandry, composting, landfill operations, and waste water treatment and is also associated with faeces, flatus and oral malodor (halitosis). A diverse range of microorganisms drives the production and degradation of methanethiol, including its aerobic and anaerobic metabolism. MT producing and degrading organisms are known to be present in terrestrial, freshwater and marine environments but may also be important in association with plant and animal (and human) hosts. This chapter considers the role of methanethiol as an intermediate of the global sulfur cycle and discusses current knowledge of microbial pathways of methanethiol production and degradation.

Introduction

Methanethiol (MT), also known as methylmercaptan, is a one-carbon organic sulfur compound with the formula CH_3SH . With a boiling point of 6°C it is a colourless gas at room temperature characterised by a pungent and disagreeable odour that has been likened to rotten cabbage. As a product of decaying biomass, it can be found widely in the environment, for instance as a compound emitted from rotting fruit and vegetables but it is also found in association with humans and animals where it is a component of the smell of faeces, flatus and can be associated with bad breath (halitosis) (NCBI Pubchem database, n.d.). The human sense of smell has a low threshold for detection of MT at 1-2 p.p.b. (Devos et al., 1990), which is exploited in its use as an additive to natural gas distribution systems in order to facilitate the detection of leaks. A leak of an unspecified quantity of MT from a chemical factory in northern France in 2013 illustrated the potential of MT as a significant malodour. A bad smell was reported in parts of northern France and as far away as across the Channel in parts of Southern England, due to the chemical having been dispersed by the wind (Reuters, 2013).

Environmental concentrations, production and degradation of MT

Environmental concentrations of MT

Only relatively few studies have measured MT concentrations in seawater, anoxic environments and industrial settings. A detailed study of production of volatile sulfur compounds in anoxic freshwater sediments in a peat bog by Lomans (Lomans et al., 1997) showed that MT was one of the dominant volatile sulfur compounds in anoxic sediments. MT concentrations ranged from 3-76 nM and its production pathway was

biological as shown by heat killed controls. Accumulation of MT in sediment slurries to which bromoethanesulfonate had been added, suggested that methanogens were mainly responsible for MT removal in these freshwater sediments. Surface freshwater MT concentrations in the peat bog were 1-8 nM and in a similar range to those of H₂S, DMS and CS₂, demonstrating that MT contributes to emission of S from such freshwater environments (Lomans et al., 1997).

In marine environments, MT concentrations have been reported to be in the range of 0.02-2 nM. In a study primarily reporting carbonyl sulfide (COS) emissions from the Aegean Sea, MT concentrations detected at time zero in incubation experiments with natural seawater samples were in a range of 50-500 pM and it was suggested that MT was subject to photodegradation and could potentially be a precursor for COS photoproduction (Ulshöfer et al., 1996). Measurements of MT were also reported for a sample transect of the Atlantic by Kettle and colleagues, who reported average MT concentrations in surface seawater of 420 pM, with localised higher concentrations up to ~1700 pM in the North African upwelling area, around ~1500 pM in a coastal region close to Montevideo and up to ~1000 pM on a transect between Montevideo and the Falkland Islands (Kettle et al., 2001). In addition to above mentioned photodegradation, the reaction of MT with sulfate, dissolved organic matter (DOM) and trace metals to form sulfate-DOM-metal complexes has also been suggested as an abiotic degradation pathway (Kiene et al., 2000).

Biological Production of MT

Biological MT production is well-known as an intermediate in metabolism of dimethylsulfoniopropionate (DMSP) (Kiene et al., 2000; Kiene and Taylor, 1988;

Reisch et al., 2011a; Reisch et al., 2011b) and DMS (Borodina et al., 2000; De Bont et al., 1981; Pol et al., 1994; Schäfer, 2007; Suylen and Kuenen, 1986), which itself can be produced through microbial degradation of DMSP (Curson et al., 2011b). In marine, estuarine and salt marsh environments, dimethylsulfoniopropionate (DMSP), an abundant metabolite released by phytoplankton and macroalgae, acts as a key precursor of DMS. Degradation of dissolved DMSP releases DMS by the activity of DMSP-lyases (Johnston et al., 2008), which are found in many aerobic and anaerobic organisms (Curson et al., 2011a; Curson et al., 2012; Curson et al., 2011b; Todd et al., 2009; Todd et al., 2011; Sun et al., 2016). Once produced, DMS is degraded to MT by a DMS monooxygenase, which was reported in some strains of *Hyphomicrobium* and *Arthrobacter* (Boden et al., 2011; Borodina et al., 2000; De Bont et al., 1981).

MT production also occurs as an intermediate of microbial DMSP degradation via the demethylation pathway. In a study of the sulfur metabolism of several isolates from the abundant marine roseobacter clade, Gonzales noted the ability of several strains to produce MT from DMSP and other precursors including DMS, DMSO, 3-methylmercaptopropionate (MMPA) and α -ketomethiol-butyrate (González et al., 1999). Subsequent characterization of the metabolic pathway producing MT from DMSP showed that, in this pathway, DMSP is initially demethylated to MMPA by DMSP demethylase encoded by *dmdA*, which was first discovered in the marine roseobacter *Ruegeria pomeroyi* DSS-3 (Howard et al., 2006). Subsequently, Reisch et al. (2011) demonstrated that after the demethylation step, MMPA is catabolised first to methylthioacryloyl-CoA (MTA-CoA) and then to MT via the demethiolation pathway (Reisch et al., 2011b). The genes designated as *dmdB*, *dmdC* and *dmdD* were shown

to encode the enzymes catalysing the transformation from MMPA to MT in *R. pomeroyi*. The presence of these genes (with the exception of *dmdD*) in the genome of ubiquitous marine bacterium *Pelagibacter ubique*, *Pelagibacter* HTCC1062 and *Ruegeria lacuscaerulensis* as well as in marine metagenomes reiterate the significance of this pathway in the marine environment (Reisch et al., 2011b; Sun et al., 2016). Sun and colleagues also demonstrated that eight *Pelagibacterales* genomes contain homologues of the *dmdABC* genes, but not *dmdD*, but *Pelagibacter* is still able to produce MT from DMSP. This suggests that the MT formation from MMPA is widespread in marine ecosystems, however a novel enzyme may be catalysing the last step of MT production from DMSP in some marine bacteria.

It was also noted that several aerobic bacteria from the genera *Corynebacterium*, *Rhizobium*, *Flavobacterium*, *Erwinia*, *Aeromonas*, *Pseudomonas* and *Yersinia* isolated from soil, sediment and marine algae cultures have the capacity to methylate hydrogen sulfide to produce MT. The activity of a thiol S-methyltransferases was demonstrated in distinct fractions of crude cell free extracts of *Pseudomonas fluorescens* PF4 subjected to gel-filtration and ion-exchange chromatography; S-adenosylmethionine was identified as a methyl donor (Drotar et al., 1987). The authors speculated that further methylation of the product MT to DMS would be a possibility and might be carried out by the same enzyme, but they did not observe DMS production in their activity assays, noting that this would depend on the K_m of the second methyl transfer reaction (Drotar et al., 1987).

Anaerobic mechanisms

In anaerobic soils and sediments, MT is primarily produced via the degradation of sulfur containing amino acids and methylation of sulfide (Lomans et al., 2002). Degradation of methionine was shown to lead to formation of MT in anoxic lake sediments (Zinder and Brock, 1978). Similarly, in anoxic salt marsh sediments, addition of methionine and S-methyl cysteine led to production of MT, less MT production was noted from DMSP (Kiene and Capone, 1988). The methyl-thiol group of methionine is cleaved (demethiolation) via the methionine-gamma-lyase enzyme (MegL), which has been purified and characterised from various bacteria such as *Pseudomonas*, *Brevibacter* and *Trichomonas* species (Bentley and Chasteen, 2004). Another route to anaerobic MT production is by the activity of thiol S-methyltransferases, which transfer methyl groups from S-adenosylmethionine to sulfide resulting in MT formation or methylate MT to form DMS (Bentley and Chasteen, 2004).

Methoxylated aromatic compounds are another precursor from which MT is produced in soil and sediments. Bak et al. (1992) isolated two anaerobic homoacetogenic species from the genus *Pelobacter* that produce MT during growth on trimethoxybenzoate or syringate by transferring the methyl group of the aromatic ring to hydrogen sulfide via thiol S-methyltransferase (Bak et al., 1992). Several anaerobic isolates have been described including members of the genera *Holophaga*, *Sporobacter*, *Sporobacterium* and *Parasporobacterium* that can methylate sulfide to MT during the degradation of aromatic methoxylated compounds (Kreft and Schink, 1993; Grech-Mora et al., 1996; Mechichi et al., 1999; Lomans et al., 2001). This

process is suggested to take place at aerobic/anaerobic interfaces of organic rich freshwater sediments as methoxylated aromatic compounds are produced during the degradation of lignin, an abundant biopolymer (Lomans et al., 2002).

Microbial degradation of MT

The main sink for MT in the environment is its degradation by microorganisms. Owing to the toxicity and foul odour of MT, only few studies have actually attempted to enrich and grow microorganisms on MT as a sole source of carbon and energy source. Therefore, most of what is known about microbial MT degradation is based on isolates in which MT is degraded as an intermediate of the metabolism of other organic sulfur compounds.

Aerobic mechanisms

Aerobic degradation of MT was shown in *Hyphomicrobium* sp. S, which was obtained from soil using DMSO as the enrichment substrate (De Bont et al., 1981). Following this, a wide range of aerobic bacteria that degrade MT have been isolated from several environments including soil, peat biofilter, lake and marine sediments, seawater and marine algal cultures. These methylotrophic and sulfur-oxidising species were affiliated with the genera *Hyphomicrobium*, *Thiobacillus*, *Rhodococcus* and *Methylophaga* (De Bont et al., 1981; Suylen et al., 1987; Cho et al., 1991; Gould and Kanagawa, 1992; Visscher and Taylor, 1993a; Pol et al., 1994; Borodina et al., 2000; Schäfer, 2007; Boden et al., 2010). Recently, *Methylotenera mobilis* JLW8 was shown to degrade MT as the sole carbon and energy source (Carrión et al., 2017).

Aerobic bacteria degrade MT by methanethiol oxidase (MTO). This enzyme has been purified from *Hyphomicrobium* and *Thiobacillus* species and shown to degrade MT to formaldehyde, hydrogen sulfide and hydrogen peroxide (Gould and Kanagawa, 1992; Suylen et al., 1987). Recently, the MTO enzyme in *Hyphomicrobium* sp. VS has been characterised in more detail, showing that it requires Cu for activity and suggesting presence of a tryptophan-tryptophylquinone (TTQ) co-factor. The identification of the encoding gene revealed that MTO it is a homologue of the so-called selenium-binding protein family whose function had previously been poorly constrained (Eyice et al., 2018). However, identification and characterisation of the human form of selenium-binding protein SELENBP1 by Pol and colleagues demonstrated that the human homologue is also a methanethiol oxidase and that a genetic defect in this gene is the underlying cause of extra-oral, or blood-borne, halitosis (Pol et al., 2018).

Genes encoding MTO are present in genomes of a wide range of microorganisms known to degrade DMS (e.g. *Hyphomicrobium* and *Thiobacillus* spp.), DMSP (*Ruegeria pomeroyi* and other roseobacter clade bacteria), and indeed a number of methanotrophic and methylotrophic bacteria. Detection of *mtoX* in metagenomes as well as the application of specific PCR primers for *mtoX* demonstrated that *mtoX* and thus MT-degrading bacteria are present in a wide range of marine and terrestrial environments. Application of the stable isotope probing method with ¹³C-labelled DMS has also been used indirectly to identify active MT-degraders in soil and lake sediment samples (Eyice et al., 2018). MT oxidases with different molecular weights to that found in *Hyphomicrobium* sp. VS have been reported in *Rhodococcus* and *Thiobacillus* strains, suggesting that other methanethiol oxidases may yet have to be

characterized in detail at the biochemical and genetic level (Kim et al., 2000; Lee et al., 2002).

An alternative sink for MT removal in aerobic environments is MT-dependent DMS production. A recent study demonstrated that MT can be methylated to DMS in aerobic bacteria through the activity of a membrane bound methyltransferase encoded by the gene *mddA* (Carrion et al., 2015). The *mddA* gene was found widely distributed in phylogenetically diverse bacteria and several isolates tested showed that the presence of the *mddA* gene correlated with the ability to form DMS from MT. Based on metagenomic datasets, it was estimated that the *mddA* gene may be present in 5-76% of soil bacteria (Carrión et al., 2015). In a subsequent study, Carrion and colleagues showed that although only a small proportion of MT (~0.1%) added to a grassland soil was converted to DMS via this pathway, the soil microbial community contained a phylogenetically diverse group of bacteria, mainly *Pseudomonas*, *Acinetobacter*, *Gemmobacter*, *Phyllobacterium*, *Rhizobium*, *Ensifer* and *Sinorhizobium* that encoded *mddA*. (Carrión et al., 2017). It is notable that the gene encoding the MddA enzyme can be found abundantly in metagenomes, particularly in soils. The relatively low conversion of MT to DMS observed in a grassland soil in the study could be due to competing pathways of MT and DMS removal and the environmental significance of this pathway requires additional analysis (Carrión et al., 2017).

Anaerobic mechanisms

DMS and MT degradation by microorganisms has been studied in several ecosystems, yet, our knowledge on microbial populations that degrade MT in anoxic environments is very limited.

MT is primarily used by methane-producing archaea (methanogens) and sulfate-reducing bacteria (SRB) in anoxic marine, freshwater and terrestrial ecosystems (Figure 1; Zinder and Brock, 1978; Lomans et al., 1999b). The first study that showed that MT is degraded to methane and carbon dioxide was carried out on samples from Lake Mendota (Wisconsin, USA) using radiolabelled ^{14}C -methyl-methionine (Zinder and Brock, 1978). However, pure methanogenic species that could utilise MT as the carbon and energy source were not isolated from the samples. Later, Kiene et al. (1986) demonstrated that methane is produced in sediment samples from a variety of habitats including freshwater, alkaline and hypersaline lakes as well as estuarine salt marshes. They also obtained, from an estuarine salt marsh sediment, the first methanogenic strain, which was capable of metabolising MT as the sole source of carbon and energy, yet did not identify this strain (Kiene et al., 1986). Following this, Ni and Boone (1991) identified the first pure methanogenic strain (*Methanolobus siciliae*) from oilfield water samples using DMS as the substrate (Ni and Boone, 1991). The cultures were subsequently shown to use MT as the catabolic substrate (Ni and Boone, 1993; Table 1). Another methanogenic strain, *Methanosarcina* sp. MTP4, was isolated from marine sediment using MT as the sole carbon and energy source (Finster et al., 1992; Table 1). The biochemical and genetic basis of methanethiol-dependent methanogenesis of *Methanosarcina* spp. has recently been identified. Three fused

methyltransferase-corrinoid enzymes (MtsD, MtsF and MtsH) were shown by mutational analysis to be involved in formation of DMS as a metabolic intermediate during carboxidotrophic growth of *Methanosarcina acetivorans*. Further characterisation indicated that these were also required for methylotrophic growth of *M. acetivorans* on DMS (Oelgeschläger and Rother, 2009). Work by Fu and Metcalf (2015) further showed that MtsF, which was highly up-regulated during growth on MT, and MtsH were capable of transferring the methyl group from MT to coenzyme M, the latter also seemed to accept DMS as a substrate (Fu and Metcalf, 2015). Other methanogens that can grow on methylated sulfur compounds have been described which appear to be obligately methylotrophic. *Methanomethylovorans hollandica* DMS1 is a MT-degrading methanogen that has been isolated from the sediment of a eutrophic freshwater pond in Nijmegen, The Netherlands, using a chemostat, which enabled high DMS degradation rates by removing inhibitory metabolites (i.e. hydrogen sulfide) (Lomans et al., 1999a). A closely related strain, *Methanomethylovorans uponensis*, was obtained from a wetland sediment and shared the ability to grow using methylated sulfur compounds MT and DMS (Cha et al., 2013). Methanogens related to *Methanomethylovorans hollandica* as well as *Methanlobus* were also present in a lab-scale bioreactor able degrade 6mM MT in the inflowing medium which had been inoculated with sludge from a paper pulp mill wastewater treatment plant (de Bok et al., 2006). Further *Methanlobus* isolates (strains WR1 and SODA) were obtained by van Leerdam and colleagues from bioreactors treating MT under alkaline conditions at pH of 8 and above and pH10, respectively, and were shown to grow on MT as sole carbon source (van Leerdam et al., 2008a; van Leerdam et al., 2008b). Additionally, a number of methanogens from the genera *Methanosarcina*, *Methanohalophilus* and *Methanlobus*, which can transform DMS to methane were isolated from anoxic

environments including alkaline lake and marine sediments (Table 1). Some of these were not tested for their growth on MT directly; however, it appears likely that due to MT being an intermediate of DMS metabolism in other methanogens, that these species can also catabolise MT.

In high-sulfate ecosystems such as marine and salt marsh sediments, SRB degrade MT to hydrogen sulfide and carbon dioxide using sulfate as the final electron acceptor (Kiene and Capone, 1988; Kiene and Visscher, 1987; Tanimoto and Bak, 1994). A relatively small number of SRB that grow on MT and sulfate have been isolated so far. These belong to the genera *Desulfotomaculum* and *Desulfosarcina*, which were obtained from laboratory scale methanogenic fermenters (Tanimoto and Bak, 1994) and mangrove sediments (Lyimo et al., 2009).

A number of studies have been carried out to understand the interaction between methanogens and SRB using 2-bromoethanesulfonic acid (BES) and molybdate as specific inhibitors of methanogenesis and sulfate reduction, respectively (Kiene et al., 1986; Lomans et al., 1997). MT was reported to be a non-competitive substrate for methanogens although competition between methanogens and SRB was observed for DMS at low substrate concentrations ($<10\ \mu\text{M}$ DMS) (Kiene et al., 1986). This microbial interaction may have significant impact on the fate of MT in the environment, particularly in marine sediments which have high sulfate concentrations.

In addition to sulfate, nitrate is also used as an electron acceptor by MT-degrading microorganisms. One example for this metabolism is *Desulfotomaculum* sp. SDN4 that was isolated from a methanogenic thermophilic fermentor and which was able to

use nitrate as terminal electron acceptor (Tanimoto and Bak, 1994). DMS-grown cells of *Thiobacillus* ASN-1, which was isolated from a *Spartina*-dominated salt marsh, were also shown to metabolise MT with nitrate and nitrite as electron acceptor (Visscher and Taylor, 1993b).

In a study that used cultivation-independent methods to identify methanogen and SRB populations degrading MT (and DMS) in the environment, Lomans and colleagues found methanogens closely related to *Methanomethylovorans hollandica* to be the dominant MT-degraders in freshwater sediments (Lomans et al, 2001). This suggests that *M. hollandica* might be a major player in the MT cycle in freshwater habitats. To our knowledge, there are as yet no studies focusing on the characterization of anaerobic MT-degrading populations using post-genomic approaches, which limits our understanding of the identity and distribution of MT-degrading microorganisms in anoxic environments.

Conclusions

The biogeochemical cycling of methylated sulfur compounds is brought about by a wide range of interconnected and interacting metabolic pathways and microorganisms. The intense study of the metabolism of DMSP and DMS during recent years has brought the role of MT as an intermediate of their microbial degradation into focus. MT is relevant as a malodorous compound in a range of industries but also in a medical context, therefore a more detailed understanding of the biochemistry, genetics and ecology of MT degrading microorganisms has considerable benefit to aid in the exploitation of the properties of microorganisms for removal of MT, and even to help understand the role of organic sulfur metabolism in plants, animals and humans. The identification of several key genes that encode

enzymes of MT metabolism facilitates a more holistic analysis of the role of diverse bacteria and archaea using metagenomics and related approaches in the future and will lead to a better understanding of MT cycling in the environment. At the same time, advances in the role of MT metabolism in human disease may only be the beginning of a better understanding how sulfur metabolism affects human health, providing an important scope to explore both host and microbiome associated pathways of MT production and degradation in more detail.

Table 1. Anaerobic archaea and bacteria that can utilise MT as a carbon and energy source.

Isolate	Isolation source	Reference
Archaea		
<i>Methanolobus siciliae</i> HI350	Oil field water	Ni and Boone (1991)
<i>Methanolobus bombayensis</i> B-1*	Marine sediment	Kadam et al. (1994)
<i>Methanolobus taylorii</i> GS-16	Estuarine sediment	Oremland and Boone (1994)
<i>Methanolobus</i> sp. strain SODA	Bioreactor treating MT at pH10	van Leerdam et al. (2008a)
<i>Methanolobus</i> sp. strain WR1	Bioreactor treating MT at pH ≥ 8	van Leerdam et al. (2008b)
<i>Methanosarcina</i> sp. MTP4	Marine sediment	Finster et al. (1992)
<i>Methanosarcina semesiae</i> MD1	Mangrove sediment	Lyimo et al. (2000)
<i>Methanomethylovorans hollandica</i> DMS1	Lake sediment	Lomans et al. (1999a)
<i>Methanohalophilus zhilinae</i> WeN5T*	Alkaline lake sediment	Mathrani et al. (1988)
<i>Methanohalophilus oregonense</i> WAL1*	Alkaline saline aquifer	Liu et al. (1990)
Bacteria		
<i>Desulfotomaculum</i> sp. MTS5	Anaerobic fermentor	Tanimoto and Bak (1994)
<i>Desulfotomaculum</i> sp. SDN4	Anaerobic fermentor	Tanimoto and Bak (1994)
<i>Desulfosarcina</i> sp. SD1	Mangrove sediment	Lyimo et al (2009)
<i>Thiobacillus</i> sp. ASN1	Salt marsh	Visscher and Taylor (1993b)

* These strains were isolated using DMS as the carbon and energy source but not tested directly for their ability to grow on MT.

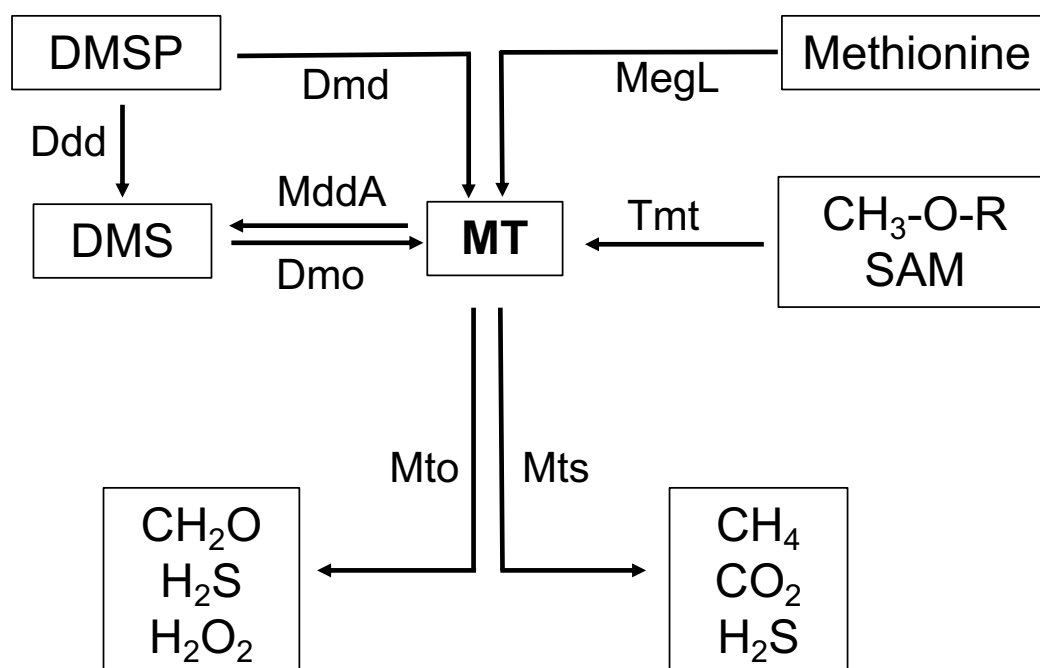


Figure 1. Simplified MT cycle and the key enzymes/pathways identified. Main MT sources include degradation of DMSP by the demethylation/demethiolation pathway (Dmd), degradation of methionine by methionine-gamma-lyase (MegL) or methyl transfer to sulfide by thiol methyltransferases (Tmt) from methoxylated aromatic compounds (CH₃-O-R) or S-adenosylmethionine (SAM). Cleavage of DMSP by DMSP-lyases (Ddd) produces DMS which can be oxidized to MT by DMS monooxygenase (Dmo). Sinks include methanethiol oxidase (Mto) which degrades MT to formaldehyde (CH₂O), hydrogen sulfide (H₂S) and hydrogen peroxide (H₂O₂), methylation of MT to DMS by methyltransferase (Mdd), and degradation in methanogens to methane (CH₄), carbon dioxide (CO₂) and hydrogen sulfide (H₂S) via activity of Mts methyltransferases.

References

- Bak, F., Finster, K., and Rothfuß, F. (1992). Formation of dimethylsulfide and methanethiol from methoxylated aromatic compounds and inorganic sulfide by newly isolated anaerobic bacteria. *Arch Microbiol* 157, 529-534.
- Bentley, R., and Chasteen, T.G. (2004). Environmental VOSCs - formation and degradation of dimethyl sulfide, methanethiol and related materials. *Chemosphere* 55, 291-317.
- Boden, R., Borodina, E., Wood, A.P., Kelly, D.P., Murrell, J.C., and Schäfer, H. (2011). Purification and characterization of dimethylsulfide monooxygenase from *Hyphomicrobium sulfonivorans*. *J Bacteriol* 193, 1250-1258.
- Boden, R., Kelly, D.P., Murrell, J.C., and Schäfer, H. (2010). Oxidation of dimethylsulfide to tetrathionate by *Methylophaga thiooxidans* sp. nov.: a new link in the sulfur cycle. *Environ Microbiol* 12, 2688-2699.
- Borodina, E., Kelly, D.P., Rainey, F.A., Ward-Rainey, N.L., and Wood, A.P. (2000). Dimethylsulfone as a growth substrate for novel methylotrophic species of *Hyphomicrobium* and *Arthrobacter*. *Arch Microbiol* 173, 425-437.
- Carrión, O., Curson, A.R.J., Kumaresan, D., Fu, Y., Lang, A.S., Mercade, E., and Todd, J.D. (2015). A novel pathway producing dimethylsulphide in bacteria is widespread in soil environments. *Nat Commun* 6, 8.
- Carrión, O., Pratscher, J., Curson, A.R.J., Williams, B.T., Rostant, W.G., Murrell, J.C., and Todd, J.D. (2017). Methanethiol-dependent dimethylsulfide production in soil environments. *ISME J* 11, 2379-2390.
- Cha, I.T., Min, U.G., Kim, S.J., Yim, K.J., Roh, S.W., and Rhee, S.K. (2013). *Methanomethylovorans uponensis* sp nov., a methylotrophic methanogen isolated from wetland sediment. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 104, 1005-1012.
- Cho, K.S., Hirai, M., and Shoda, M. (1991). Degradation characteristics of hydrogen sulfide, methanethiol, dimethyl sulfide and dimethyl disulfide by *Thiobacillus thioparus* DW44 isolated from peat biofilter. *J Ferment Bioeng* 71, 384-389.
- Curson, A., Sullivan, M.J., Todd, J.D., and Johnston, A.W.B. (2011a). DddY, a periplasmic dimethylsulfoniopropionate lyase found in taxonomically diverse species of Proteobacteria. *ISME J* 5, 1191-1200.
- Curson, A.R.J., Fowler, E.K., Dickens, S., Johnston, A.W.B., and Todd, J.D. (2012). Multiple DMSP lyases in the gamma-proteobacterium *Oceanimonas doudoroffii*. *Biogeochem* 110, 109-119.
- Curson, A.R.J., Todd, J.D., Sullivan, M.J., and Johnston, A.W.B. (2011b). Catabolism of dimethylsulphoniopropionate: microorganisms, enzymes and genes. *Nat Rev Micro* 9, 849-859.

de Bok, F.A.M., van Leerdam, R.C., Lomans, B.P., Smidt, H., Lens, P.N.L., Janssen, A.J.H., and Stams, A.J.M. (2006). Degradation of methanethiol by methanogenic archaea in a lab-scale upflow anaerobic sludge blanket reactor. *Appl Environ Microbiol* 72, 7540-7547.

De Bont, J.A.M., van Dijken, J.P., and Harder, W. (1981). Dimethyl sulfoxide and dimethyl sulphide as a carbon, sulphur and energy source for growth of *Hyphomicrobium* S. *J Gen Microbiol* 127, 315-323.

Devos, M., Patte, F., Rouault, J., Laffort, P., and Gemert, L.J. (1990). Standardized human olfactory thresholds. (IRL Press, Oxford).

Drotar, A., Burton, G.A., Jr., Tavernier, J.E., and Fall, R. (1987). Widespread occurrence of bacterial thiol methyltransferases and the biogenic emission of methylated sulfur gases. *Appl Environ Microbiol* 53, 1626-1631.

Eyice, Ö., Myronova, N., Pol, A., Carrión, O., Todd, J.D., Smith, T.J., Gurman, S.J., Cuthbertson, A., Mazard, S., Mennink-Kersten, M.A.S.H., *et al.* (2018). Bacterial SBP56 identified as a Cu-dependent methanethiol oxidase widely distributed in the biosphere. *The Isme Journal* 12, 145-160.

Finster, K., Tanimoto, Y., and Bak, F. (1992). Fermentation of methanethiol and dimethylsulfide by a newly isolated methanogenic bacterium. *Arch Microbiol* 157, 425-430.

Fu, H., and Metcalf, W.W. (2015). Genetic basis for metabolism of methylated sulfur compounds in *Methanosarcina* species. *J Bacteriol* 197, 1515-1524.

González, J.M., Kiene, R.P., and Moran, M.A. (1999). Transformation of sulfur compounds by an abundant lineage of marine bacteria in the alpha -subclass of the class Proteobacteria. *Appl Environ Microbiol* 65, 3810-3819.

Gould, W.D., and Kanagawa, T. (1992). Purification and properties of methyl mercaptan oxidase from *Thiobacillus thioparus* TK-m. *J Gen Microbiol* 138, 217-221.

Grech-Mora, I., Fardeau, M.-L., Patel, B., Ollivier, B., Rimbault, A., Prensier, G., Garcia, J.-L., and Garnier-Zarli, E. (1996). Isolation and characterization of *Sporobacter termitidis* gen. nov., sp. nov., from the digestive tract of the wood-feeding termite *Nasutitermes lujae*, *Int J Syst Bacteriol* 46, 512-518.

Howard, E.C., Henriksen, J.R., Buchan, A., Reisch, C.R., Buergmann, H., Welsh, R., Ye, W.Y., Gonzalez, J.M., Mace, K., Joye, S.B., *et al.* (2006). Bacterial taxa that limit sulfur flux from the ocean. *Science* 314, 649-652.

Johnston, A.W.B., Todd, J.D., Sun, L., Nikolaidou-Katsaridou, M.N., Curson, A.R.J., and Rogers, R. (2008). Molecular diversity of bacterial production of the climate-changing gas, dimethyl sulphide, a molecule that impinges on local and global symbioses. *J Exp Bot* 59, 1059-1067.

Kadam, P.C., Ranade, D.R., Mandelco, L., and Boone, D.R. (1994). Isolation and characterization of *Methanlobus bombayensis* sp. nov., a methylotrophic

methanogen that requires high concentrations of divalent cations. *Int J Syst Evol Microbiol* 44, 603-607.

Kettle, A.J., Rhee, T.S., von Hobe, M., Poulton, A., Aiken, J., and Andreae, M.O. (2001). Assessing the flux of different volatile sulfur gases from the ocean to the atmosphere. *J Geophys Res Atmos* 106, 12193-12209.

Kiene, R.P., and Capone, D.G. (1988). Microbial transformations of methylated sulfur compounds in anoxic salt marsh sediments. *Microb Ecol* 15, 275-291.

Kiene, R.P., Linn, L.J., and Bruton, J.A. (2000). New and important roles for DMSP in marine microbial communities. *J Sea Res* 43, 209-224.

Kiene, R.P., Oremland, R.S., Catena, A., Miller, L.G., and Capone, D.G. (1986). Metabolism of reduced methylated sulfur compounds in anaerobic sediments and by a pure culture of an estuarine methanogen. *Appl Environ Microbiol* 52, 1037-1045.

Kiene, R.P., and Taylor, B.F. (1988). Biotransformations of organosulphur compounds in sediments via 3-mercaptopyruvate. *Nature* 332, 148-152.

Kiene, R.P., and Visscher, P.T. (1987). Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate in anoxic salt marsh sediments. *Appl Environ Microbiol* 53, 2426-2434.

Kim, S.-J., Shin, H.-J., Kim, Y.-C., Lee, D.-S., and Yang, J.-W. (2000). Isolation and purification of methyl mercaptan oxidase from *Rhodococcus rhodochrous* for mercaptan detection. *Biotechnol Bioprocess Eng* 5, 465-468.

Kreft, J.-U., and Schink, B. (1993). Demethylation and degradation of phenylmethylethers by the sulfide-methylating homoacetogenic bacterium strain TMBS 4. *Arch Microbiol* 159, 308-315.

Lee, H.-H., Kim, S.-J., Shin, H.-J., Park, J.-Y., and Yang, J.-W. (2002). Purification and characterisation of methyl mercaptan oxidase from *Thiobacillus thioparus* for mercaptan detection. *Biotechnol Bioprocess Eng* 7, 375-379.

Liu, Y., Boone, D.R., and Choy, C. (1990). *Methanohalophilus oregonense* sp. nov., a methylotrophic methanogen from an alkaline, saline aquifer. *Int J Syst Evol Microbiol* 40, 111-116.

Lomans, B.P., Leijdekkers, P., Wesselink, J.-J., Bakkes, P., Pol, A., van der Drift, C., and Op den Camp, H.J. (2001). Obligate sulfide-dependent degradation of methoxylated aromatic compounds and formation of methanethiol and dimethyl sulfide by a freshwater sediment isolate, *Parasporobacterium paucivorans* gen. nov., sp. nov. *Appl Environ Microbiol* 67, 4017-4023.

Lomans, B.P., Maas, R., Luderer, R., Op den Camp, H.J., Pol, A., van der Drift, C., and Vogels, G.D. (1999a). Isolation and characterization of *Methanomethylovorans hollandica* gen. nov., sp. nov., isolated from freshwater sediment, a methylotrophic methanogen able to grow on dimethyl sulfide and methanethiol. *Appl Environ Microbiol* 65, 3641-3650.

- Lomans, B.P., Op den Camp, H.J., Pol, A., van der Drift, C., and Vogels, G.D. (1999b). Role of methanogens and other bacteria in degradation of dimethyl sulfide and methanethiol in anoxic freshwater sediments. *Appl Environ Microbiol* 65, 2116-2121.
- Lomans, B.P., Smolders, A.J.P., Intven, L.M., Pol, A., Op Den Camp, H.J.M., and Van Der Drift, C. (1997). Formation of dimethyl sulfide and methanethiol in anoxic freshwater sediments. *Appl Environ Microbiol* 63, 4741-4747.
- Lomans, B.P., van der Drift, C., Pol, A., and Op den Camp, H.J.M. (2002). Microbial cycling of volatile organosulfur compounds. *Cell Mol Life Sci* 59, 575-588.
- Lyimo, T.J., Pol, A., Harhangi, H.R., Jetten, M.S.M., and Op den Camp, H.J.M. (2009). Anaerobic oxidation of dimethylsulfide and methanethiol in mangrove sediments is dominated by sulfate-reducing bacteria. *FEMS Microbiol Ecol* 70, 483-492.
- Lyimo, T.J., Pol, A., Op den Camp, H.J., Harhangi, H.R., and Vogels, G.D. (2000). *Methanosarcina semesiae* sp. nov., a dimethylsulfide-utilizing methanogen from mangrove sediment. *Int J Syst Evol Microbiol* 50, 171-178.
- Mathrani, I.M., Boone, D.R., Mah, R.A., Fox, G.E., and Lau, P.P. (1988). *Methanohalophilus zhilinae* sp. nov., an alkaliphilic, halophilic, methylotrophic methanogen. *Int J Syst Bacteriol* 38, 139-142.
- Mechichi, T., Labat, M., Garcia, J.-L., Thomas, P., and Patel, B.K.C. (1999). *Sporobacterium olearium* gen. nov., sp. nov., a new methanethiol-producing bacterium that degrades aromatic compounds, isolated from an olive mill wastewater treatment digester. *Int J Syst Evol Microbiol* 49, 1741-1748.
- NCBI. (n.d.) PubChem Compound Database; CID=878
<https://pubchem.ncbi.nlm.nih.gov/compound/878> [accessed 16 September 2018]
- Ni, S.S., and Boone, D.R. (1991). Isolation and characterization of a dimethyl sulfide-degrading methanogen, *Methanlobus siciliae* Hi350, from an oil-well, characterization of *M. siciliae* T4/Mt, and emendation of *M. siciliae*. *Int J Syst Bacteriol* 41, 410-416.
- Ni, S.S., and Boone, D.R. (1993). Catabolism of dimethylsulfide and methane thiol by methylotrophic methanogens (London: Chapman & Hall).
- Oelgeschläger, E., and Rother, M. (2009). In vivo role of three fused corrinoid/methyl transfer proteins in *Methanosarcina acetivorans*. *Mol Microbiol* 72, 1260-1272.
- Oremland, R.S., and Boone, D.R. (1994). *Methanlobus taylorii* sp. nov., a new methylotrophic, estuarine methanogen. *Int J Syst Evol Microbiol* 44, 573-575.
- Pol, A., Op den Camp, H.J.M., Mees, S.G.M., Kersten, M.A.S.H., and van der Drift, C. (1994). Isolation of a dimethylsulfide-utilizing *Hyphomicrobium* species and its application in biofiltration of polluted air. *Biodegr* 5, 105-112.

Pol, A., Renkema, G.H., Tangerman, A., Winkel, E.G., Engelke, U.F., de Brouwer, A.P.M., Lloyd, K.C., Araiza, R.S., van den Heuvel, L., Omeran, H., *et al.* (2018). Mutations in SELENBP1, encoding a novel human methanethiol oxidase, cause extraoral halitosis. *Nature Genet* 50, 120-129.

Reisch, C.R., Moran, M.A., and Whitman, W.B. (2011a). Bacterial catabolism of dimethylsulfoniopropionate (DMSP). *Front Microbiol* 2, 172.

Reisch, C.R., Stoudemayer, M.J., Varaljay, V.A., Amster, I.J., Moran, M.A., and Whitman, W.B. (2011b). Novel pathway for assimilation of dimethylsulphoniopropionate widespread in marine bacteria. *Nature* 473, 208-211.

Reuters (2013). Rotten eggs stench reaches UK after French gas leak. <https://www.reuters.com/article/us-france-gasleak-idUSBRE90L03M20130122> [accessed 16 September 2018].

Schäfer, H. (2007). Isolation of *Methylophaga* spp. from marine dimethylsulfide-degrading enrichment cultures and identification of polypeptides induced during growth on dimethylsulfide. *Appl Environ Microbiol* 73, 2580-2591.

Sun, J., Todd, J.D., Thrash, J.C., Qian, Y., Qian, M.C., Temperton, B., Guo, J., Fowler, E.K., Aldrich, J.T., Nicora, C.D., *et al.* (2016). The abundant marine bacterium *Pelagibacter* simultaneously catabolizes dimethylsulfoniopropionate to the gases dimethyl sulfide and methanethiol. *Nature Microbiology* 1, 16065.

Suylen, G.M.H., and Kuenen, J.G. (1986). Chemostat enrichment and isolation of *Hyphomicrobium* EG a dimethyl sulfide oxidizing methylotroph and reevaluation of *Thiobacillus* MS1. *Antonie Van Leeuwenhoek* 52, 281-293.

Suylen, G.M.H., Large, P.J., Vandijken, J.P., and Kuenen, J.G. (1987). Methyl mercaptan oxidase, a key enzyme in the metabolism of methylated sulfur-compounds by *Hyphomicrobium* EG. *J Gen Microbiol* 133, 2989-2997.

Tanimoto, Y., and Bak, F. (1994). Anaerobic degradation of methylmercaptan and dimethyl sulfide by newly isolated thermophilic sulfate-reducing bacteria. *Appl Environ Microbiol* 60, 2450-2455.

Todd, J.D., Curson, A.R.J., Dupont, C.L., Nicholson, P., and Johnston, A.W.B. (2009). The dddP gene, encoding a novel enzyme that converts dimethylsulfoniopropionate into dimethyl sulfide, is widespread in ocean metagenomes and marine bacteria and also occurs in some Ascomycete fungi. *Environ Microbiol* 11, 1376-1385.

Todd, J.D., Curson, A.R.J., Kirkwood, M., Sullivan, M.J., Green, R.T., and Johnston, A.W.B. (2011). DddQ, a novel, cupin-containing, dimethylsulfoniopropionate lyase in marine roseobacters and in uncultured marine bacteria. *Environ Microbiol* 13, 427-438.

Ulshöfer, V.S., Flock, O.R., Uher, G., and Andreae, M.O. (1996). Photochemical production and air-sea exchange of carbonyl sulfide in the eastern Mediterranean Sea. *Mar Chem* 53, 25-39.

van Leerdam, R.C., Bonilla-Salinas, M., de Bok, F.A.M., Bruning, H., Lens, P.N.L., Stams, A.J.M., and Janssen, A.J.H. (2008a). Anaerobic methanethiol degradation and methanogenic community analysis in an alkaline (pH 10) biological process for liquefied petroleum gas desulfurization. *Biotechnology and Bioengineering* 101, 691-701.

van Leerdam, R.C., de Bok, F.A.M., Bonilla-Salinas, M., van Doesburg, W., Lomans, B.P., Lens, P.N.L., Stams, A.J.M., and Janssen, A.J.H. (2008b). Methanethiol degradation in anaerobic bioreactors at elevated pH (≥ 8): Reactor performance and microbial community analysis. *Bioresource Technology* 99, 8967-8973.

Visscher, P.T., and Taylor, B.F. (1993a). A new mechanism for the aerobic catabolism of dimethyl sulfide. *Appl Environ Microbiol* 59, 3784-3789.

Visscher, P.T., and Taylor, B.F. (1993b). Aerobic and anaerobic degradation of a range of alkyl sulfides by a denitrifying marine bacterium. *Appl Environ Microbiol* 59, 4083-4089.

Zinder, S.H., and Brock, T.D. (1978). Methane, carbon dioxide, and hydrogen sulfide production from the terminal thiol group of methionine by anaerobic lake sediments. *Appl Environ Microbiol* 35, 344-352.